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Effect of glyphosate on the foraging activity of the European honey bee (*Apis mellifera* L.)

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Abstract: Glyphosate is a widely used agrochemical. Nevertheless, only a few studies have investigated its effect on bees, specifically its influence on their foraging activity. This article provides a summary of the prominent research results on this issue, published in journals in the field of experimental biology. The effect of commonly used concentrations of glyphosate on honey bee navigation has been evaluated in several studies, as well as concentrations that are reportedly sublethal. Exposure to this herbicide increases the flight time back to the hive and affects the flight trajectories of these bees. These results imply that glyphosate at certain concentrations reduces their sensitivity to nectar rewards in associative memories. The contact of bees with non-lethal concentrations of glyphosate results in sublethal effects that affect foraging. In the future, the behaviour of glyphosate and its effect on bees in their natural environment need to be explored.

Keywords: plant protection; pollinator; bee memory; bee orientation; sugar syrup

The production of sufficient food for the growing global population is a challenge for agriculture (Rossi et al. 2020). With increased agricultural activities, the use of different pesticides to protect production has also increased without considering the consequences. Pesticides simultaneously affect human health and the environment (Jolodar et al. 2021). As their environmental impact is broad, we focused only on one aspect significantly affected by pesticides: the European honey bee, *Apis mellifera* L.

Honey bees pollinate 35% of the world's cultivated crops (Aizen et al. 2008) and approximately 130 plant species (Kaplan 2008). Honey bees are essential for human life and health, and the consequences of a decline in their populations have often been dis-

cussed. Using the standardised Prevention of Honey Bee Colony Losses (COLOSS) questionnaire in 35 countries, Gray et al. (2020) reported the loss rates of a managed honey bee colony during the winter of 2018–2019. Colony collapse disorder (CCD) is characterised by the loss of workers from the hive (Lu et al. 2012, Atanasov et al. 2021). These conditions have also been reported by Kulinčević et al. (1982).

Bommuraj et al. (2021) stated that along with adversities, such as the presence of *Varroa destructor*, microbial and viral pathogens, malnutrition, habitat loss, and migratory stress, increased pesticide application is also a primary cause of CCD (Glinski et al. 2012, Bommuraj et al. 2021).

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Honey bees bring pesticide residues to hives from the environment through pollen, nectar, water, honeydew, or propolis (Mullin et al. 2010, Bommuraj et al. 2021, Wilmart et al. 2021). Residues of insecticides, fungicides, and herbicides are present in honey bees and hive matrices. Bees are characterised by the presence of sublethal doses of the aforementioned substances (Lambert et al. 2013, Peghaire et al. 2020).

Bees have high market value, one of the main reasons why the unfamiliar effects of fungicides and herbicides on these insects have been extensively studied (Belsky and Joshi 2020, United States Environmental Protection Agency 2017). Herbicides are not evaluated for toxicity to bees because no active exposure to these pollinators is expected. Our review focused on these issues.

Herbicides. Herbicides used to control weeds have different modes of action. They can influence photosynthesis, amino acid and lipid biosynthesis, growth and cell division (Shefali et al. 2021).

First-generation herbicides adversely affect the environment and human health owing to their high persistence and off-target toxicity. Owing to the impacts on human and environmental health, the development of new herbicides has focused on target specificity, high selectivity, low toxicity, low application rates, and economic and environmental friendliness (Qu et al. 2021).

Herbicide resistance is a global problem (Brankov et al. 2021, Qu et al. 2021). One element of an integrated weed control system is crop rotation, which leads to the possibility of applying herbicides with different modes of action, thereby limiting the development of resistance (Brankov et al. 2021).

Exposure assessment of herbicides to bees and humans. Some of the selective herbicides targeting dicotyledonous weeds and graminicides targeting grass weeds have long-term residual effects. They can contaminate bees that forage on flowering weeds tolerant to herbicides in treated areas (Zioga et al. 2020). Bees are exposed to pesticides in many ways (Krupke et al. 2012), and their hives are contaminated by these chemicals through nectar or pollen collection (Pohorecka et al. 2012, Goñalons and Farina 2018) or water gathering (Goñalons and Farina 2018). After returning to the hive, a forager can contaminate its mates through body contact, food, or sharing of collected resources (Grüter and Farina 2007). Other sources of contamination include herbicide-exposed flowering perennial herbs, shrubs, and trees in the landscape. They may not show a visible herbicidal effect but can easily contaminate

bees. Herbicides not classified as dangerous to bees can be used without limits (Zioga et al. 2020).

Glyphosate has also caused several societal, political, scientific, and legal disputes. The European Food Safety Authority, European Chemicals Agency, International Agency for Research on Cancer, and Monsanto are involved in research on glyphosate carcinogenicity (Morvillo 2020).

Glyphosate characteristics and usage. Glyphosate, [N-(phosphonomethyl) glycine], is a non-selective herbicide (Goldsborough and Brown 1988, Richmond 2018, Muñoz et al. 2021) with a molecular formula of $C_3H_8NO_5P$ and a molecular weight 169.1 g/mol. Glyphosate usage has extended beyond agriculture, and these herbicides are used in gardening, forest engineering, illegal crop control, and public transportation roads (Giesy et al. 2000).

This herbicide was first synthesised in 1950 and patented as a chemical chelator capable of binding to metals such as calcium, magnesium, and manganese. Owing to its magnesium binding capacity, glyphosate can inhibit plant and bacterial enzymes (Richmond 2018).

On a large scale, glyphosate is produced as a glyphosate salt mixed with co-adjuvant and inert compounds. Its commercial name Roundup® was first introduced in 1970 by Monsanto (Villamar-Ayala et al. 2019) and introduced to the market in 1974 (Faghani and Rahimian 2018, Muñoz et al. 2021).

Between 1995 and 2014, the global use of glyphosate increased from 7–16 million to 126 million kg. Glyphosate pesticides have become some of the most widely used and sold pesticides (60% of the total sales) (Zhang et al. 2011, Balbuena et al. 2015, Villamar-Ayala et al. 2019).

Glyphosate is typically administered by spraying it directly on foliage (Giesy et al. 2000). The herbicide may remain on crops after its long-term application (Zhang et al. 2011). Contamination of honey by glyphosate residues and its metabolite aminomethylphosphonic acid has been detected in different countries, such as the United States of America (USA), Switzerland, Uruguay, and Canada (De Souza et al. 2021).

Microbial transformation and mineralisation are methods of rapid degradation. Different catabolic pathways have been employed by microbial biodegrades (Sviridov et al. 2015). Tran et al. (2017) described the electrochemical oxidation of glyphosate.

Glyphosate and bees. Several laboratory and field studies were conducted to determine the potential

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toxicity of this pesticide. Giesy et al. (2000) demonstrated no acute or chronic adverse effects on honey bees when good agricultural practices are followed.

However, the sublethal effects of glyphosate on non-target organisms were scarcely evaluated (Herbert et al. 2014, Thompson et al. 2014). Glyphosate exposure at sublethal doses reduced the sensitivity and decreased associative memory in bees (De Souza et al. 2021).

The effects of different glyphosate concentrations on bees after 96 h were verified by Lima et al. (2019). Thirty Africanised bees consumed Roundup® at doses of 0.0, 0.5, 1.0, 1.5, 5.0, and 10.0 g in 100 mL of a 50% aqueous sucrose solution placed in a container inside cylindrical polyvinyl chloride cages. The number of dead bees observed at each dose of glyphosate followed a binomial distribution with the following parameters: $n(i)$, the number of bees per cage; and p_i , the mortality rate. The estimates for both parameters were significant and did not differ significantly between the a priori methods used. A lethal dose value of 50% of 1.57 g of Roundup® was determined (Lima et al. 2019).

Previous studies have reported chronic adverse effects of glyphosate exposure on bee behaviour (Herbert et al. 2014). Cage experiments by Faghani and Rahimian (2018) indicated the contribution of glyphosate herbicides to CCD. The authors demonstrated that bees fed with Roundup®-supplemented feed exhibited higher mortality rates.

Bee microflora, and thus immunity and tolerance to pathogens, can be disrupted by glyphosate, which reduces beneficial bacterial species (Dai et al. 2018, Motta et al. 2018, Blot et al. 2019, Motta and Moran 2020).

Foraging activity of bees and glyphosate. Honeybees are an accurate biosensor for environmental pollutants. Their appetitive behavioural response is a suitable tool to test the sublethal effects of agrochemicals (Herbert et al. 2014, Goñalons and Farina 2018). Foraging behaviour relies on learning and remembering processes (Farina et al. 2019). Elementary associative learning implies that bees learn a specific connection between a floral smell and a reward and strengthen this association through different foraging events (Menzel 1999, 2012).

Forager bees prefer food sources with low glyphosate concentrations. However, if honey bees continue foraging for glyphosate-contaminated sources, the exposure is repeated at each revisit. Honeybees establish predictive relationships between events that

occur concurrently in their environment and learn which stimuli are relevant through associative learning (Farina et al. 2019).

Herbert et al. (2014) reported that glyphosate herbicides affect flight patterns, foraging behaviour, homing time, and appetite. Using an artificial feeder with a solution of sucrose and glyphosate and by monitoring the foraging variables of bees, the effects of acute exposure to glyphosate were investigated. They also indicated that glyphosate at concentrations found in agroecosystems could reduce the sensitivity to nectar rewards and impair associative learning in honey bees. These results are consistent with those of Goñalons and Farina (2018). The authors found no effect on foraging behaviour. Bees returning to the hive could thus become a source of pesticide contamination to the hive (Herbert et al. 2014). They also stated that acute exposure to sublethal glyphosate concentrations during olfactory proboscis extension response conditioning decreases the short-term memory of bees and impairs more complex forms of associative learning.

Pesticides affect the accuracy of bee foraging and survival through their effects on learning and memory (Henry et al. 2012, De Stefano et al. 2014, Karahan et al. 2015, Zaluski et al. 2015). Honey bees fed sugar syrups with higher glyphosate concentrations exhibited more indirect flights. The return of foragers to the hive, namely, navigation, is negatively affected by the oral consumption of glyphosate at a concentration usually employed by common agricultural practices (Belsky and Joshi 2020).

Luo et al. (2021) tested the ability of bees to associate smell with rewards and remember this association after treatment with contaminated food. Exposure to Roundup® for 11 days at 1/2- and 1-times the common concentration led to significant memory impairment. This result differs from those of the experiments conducted by Herbert et al. (2014).

Delkash-Roudsari et al. (2020) tested the impact of Roundup® on honey bees. The authors reported the adverse effects of a single dose of imidacloprid and ethionine on the learning and movement of bees. According to these authors, glyphosate did not affect learning, and the movement of bees was affected to a lesser extent, whereas at a certain amount, Roundup® impacted their circadian rhythm. Chronic exposure to glyphosate can affect the success of pollination.

Balbuena et al. (2015) tested the effects of commonly used concentrations of glyphosate in agriculture (Giesy et al. 2000), as well as two additional

sublethal concentrations, on honey bee navigation. They proposed that honey bees collecting nectar with trace amounts of glyphosate may have difficulty in integrating complex but essential-for-navigation information from the flight radius. They used a catch-and-release method wherein pollinators flying to the hive were displaced during foraging trips. Thus, the effects of sublethal doses of glyphosate on the orientation and navigation of bees could be assessed.

Balbuena et al. (2015) suggested that the contact of bees with commonly used doses of glyphosate worsens the cognitive capacities of bees required to retrieve and integrate spatial information for their successful return to the hive. These results showed that after exposure, the bees started flying immediately in a straight path from the release site (Figures 1A, B) or showed fewer regular flights (Figure 1C). Exposure to higher concentrations of glyphosate further impaired the navigation ability of bees. Bees fed with 10 mg/L

of glyphosate required more time to fly home directly and took more indirect flights after the second release than bees treated with lower glyphosate concentrations. Balbuena et al. (2015) stated that honey bee navigation is affected by the ingestion of glyphosate residues, with potential long-term negative consequences on the success of colony foraging.

DISCUSSION

Glyphosate residues are a risk for insects, as well as the entire environment. In countries that have introduced glyphosate-tolerant crops, traces of glyphosate have been detected in honey (Rubio et al. 2014), air particles, and rain samples (Chang et al. 2011, Alonso et al. 2014). Glyphosate residues have also been found on the surface of water sources close to fields that may have been visited by bees and are treated with agricultural chemicals (Balbuena et al. 2015).

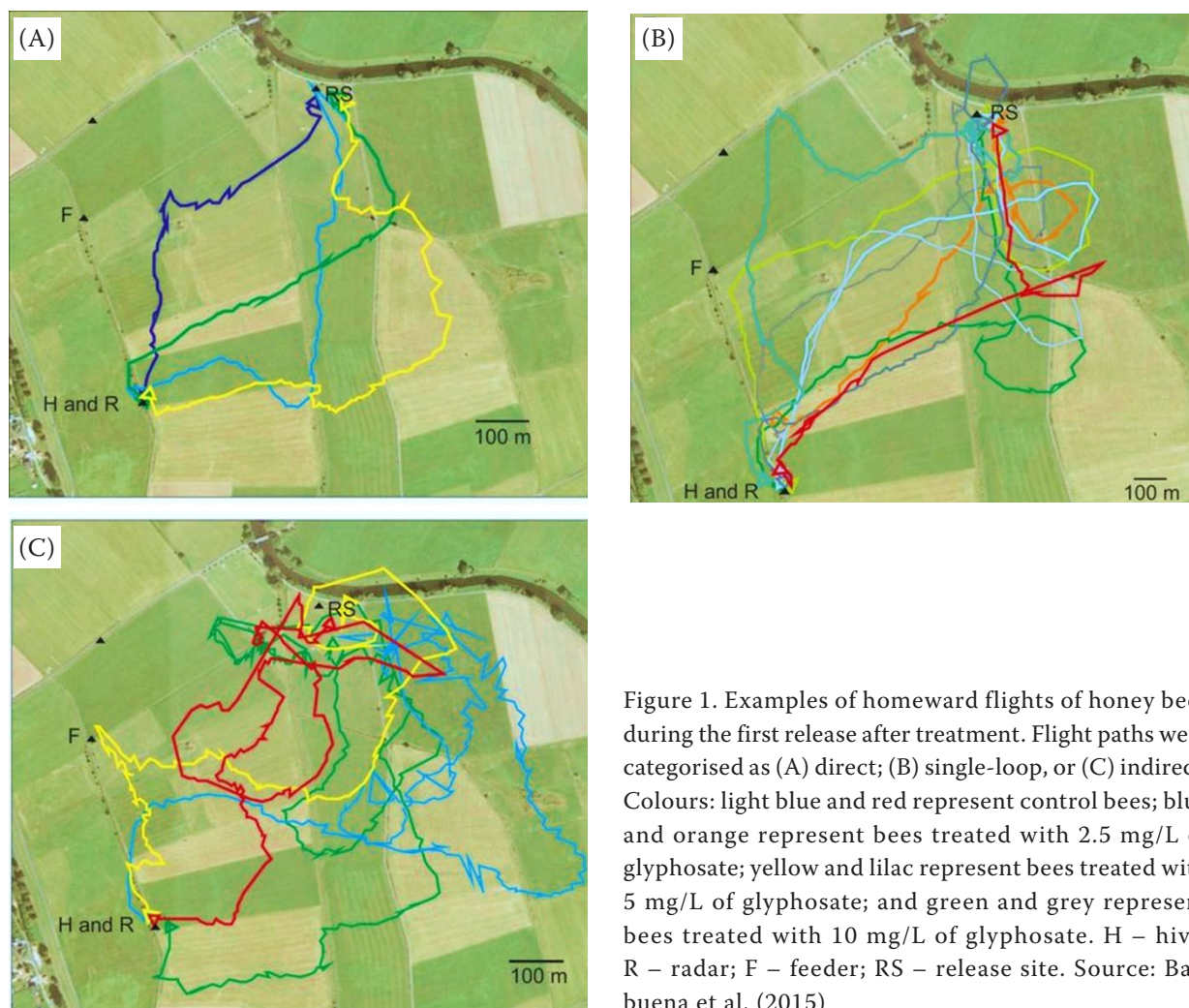


Figure 1. Examples of homeward flights of honey bees during the first release after treatment. Flight paths were categorised as (A) direct; (B) single-loop, or (C) indirect. Colours: light blue and red represent control bees; blue and orange represent bees treated with 2.5 mg/L of glyphosate; yellow and lilac represent bees treated with 5 mg/L of glyphosate; and green and grey represent bees treated with 10 mg/L of glyphosate. H – hive; R – radar; F – feeder; RS – release site. Source: Balbuena et al. (2015)

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The risk of ingesting this herbicide by bees is not as significant and long-term as that of selective herbicides because total herbicides destroy all vegetation. However, glyphosate can pose a risk to pollinators within a few days of its effects on crops and nearby flowering weeds.

According to Herbert et al. (2014), lower sensitivity to reward, formation of weak associative memories that can be quickly extinguished, and difficulty in forming non-elemental associations are the result of sublethal doses of exposure of glyphosate to bees.

Balbuena et al. (2015) added that glyphosate impairs the use of stored information about the environment acquired during exploratory orientation flights of foragers and the experience gained from homing flights throughout the experiment. Faita et al. (2018) reported that glyphosate could also alter the structure of royal jelly-producing glands. This could damage bee development and, thus, negatively affect the survival of bee colonies.

The results for short-term memory obtained by Herbert et al. (2014) are different from those of Luo et al. (2021). Luo et al. (2021) stated that long-term exposure to sublethal glyphosate levels did not affect the establishment of short-term memory (15 min) but hindered the ability of bees to establish links between smell and reward.

Differences between the data published by Luo et al. (2021) and Herbert et al. (2014) were also used to assess the effect of glyphosate on insect movement. A higher concentration of Roundup® decreased the climbing ability of insects (Luo et al. 2021). Herbert et al. (2014) reported no changes in the locomotive and directional activity of honey bees exposed to glyphosate at concentrations of 2.5 and 5 mg/L for 15 days.

The effect of Roundup® on locomotion has also been observed in other insects and soil invertebrates. Michalkova and Pekar (2009) explored the speed of locomotion of *Pardosa* spiders and the crawling speed of *Poecilus* beetles. Janssens and Stoks (2017) investigated the foraging activity or swimming speed of damselfly larvae exposed to Roundup®. Roundup®-inhibited locomotion on *Caenorhabditis elegans* was discussed by García-Espíñeira et al. (2018).

According to Abraham et al. (2018), farmers often double the herbicide concentration to address resistance. Thus, evaluating the effect of actual applications of commercially formulated glyphosate at the recommended concentration, rather than pure glyphosate, on honey bees is necessary. In future

studies, the behaviour of glyphosate and its effect on bees in their natural environment needs to be explored.

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